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Hepatitis C Virus Genotypes in Different Regions of the Former Soviet Union (Russia, Belarus, Moldova, and Uzbekistan)

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The prevalence of HCV genotypes in four republics of the former Soviet Union (Russia, Belarus, Moldova, and Uzbekistan) was investigated. Overall, 197 HCV isolates from 66 blood donors and 131 patients with chronic hepatitis were typed. Viral sequences from sera of infected subjects were amplified by nested RT-PCR using primers from the core region and typed by one or two techniques: (1) DNA enzyme immunoassay (DEIA) and (2) PCR with a set of type-specific primers. Only three major HCV genotypes were identified in this study population. HCV 1b was found to be the predominant virus type both among blood donors and chronic hepatitis patients, followed by 3a, 2a, and 1a (chronic hepatitis patients: 1b-82%; 3a-10%; 2a-4%, 1a-5% and 2c-1%; blood donors: 1b-77%; 3a-17%; and 2a-6%). No significant difference in genotype distribution was observed between different countries or between blood donors and chronic hepatitis patients within the same country. Results of the genotyping procedures were confirmed by direct sequencing of 216 nt PCR fragments corresponding to part of HCV core gene. Phylogenetic analysis of HCV 1b sequences from this study and from the Genbank demonstrated that the sequences from the former Soviet Union do not form evolutionary lineage(s) different from those of strains of the same subtype circulating in other geographical regions. ***J. Med. Virol. 53:36–40, 1997.***

KEY WORDS: hepatitis; HCV; genotypes

INTRODUCTION

Hepatitis C virus (HCV) is considered the major cause of parenteral and sporadic non-A, non-B hepatitis all over the world [Choo et al., 1991]. Sequence studies of different HCV isolates have demonstrated the remarkable heterogeneity of HCV genome [Bukh et al., 1995; Simmonds, 1995]. Presently, 6 major types (genotypes) have been reported, each consisting of one or more subtypes [Mellor et al., 1996; Simmonds et al., 1996; Lamballerie et al., 1997]. Some genotypes (1a, 1b, 2a, 2b, 3a) are widely distributed around the world, while the others have a more restricted distribution, such as genotype 4 to the Middle East and Africa, genotype 5a to South Africa, and genotype 6 to South-East Asia [Bukh et al., 1995; Simmonds, 1995]. There is an increasing evidence that HCV types possess different biological potentials; certain HCV genotypes are more amenable to interferon treatment and more frequently associated with severe forms of liver disease [Pozatto et al., 1991; Kanai et al., 1992; Silini and Mondelli, 1995]. Thus typing of HCV isolates becomes an additional tool in the diagnosis of HCV infection [Roggendorf et al., 1996].

Little is known about the prevalence of HCV genotypes in the former Soviet Union, a territory endemic for hepatitis C [Viazov et al., 1992; 1994]. We investigated the distribution of HCV genotypes in blood donors and chronic hepatitis patients from Russia, Belarus, and Moldova, and in chronic hepatitis patients from Uzbekistan, new independent states (NIS) formed after the collapse of the Soviet Union.

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TABLE I. HCV Genotypes in Chronic Hepatitis Patients From the Former Soviet Union

		HCV types				
Country	n	1a	1b	2a	2c	3a
Russia	45	1 (2%)	34 (76%)	3 (7%)	1 (2%)	6 (13%)
Belarus	20	1 (5%)	14 (70%)	1 (5%)	—	4 (20%)
Moldova	56	4 (7%)	50 (89%)	—	—	2 (4%)
Uzbekistan	10	—	9 (90%)	—	—	1 (10%)
Total	131	6 (5%)	107 (82%)	4 (4%)	1 (1%)	13 (10%)

MATERIALS AND METHODS

In this study, 197 subjects were enrolled; this included 66 paid or volunteer blood donors and 131 adult chronic hepatitis patients. These individuals were identified by the diagnostic RT-PCR with primers from the 5′-UTR [Widell et al., 1991] among subjects positive for anti-HCV by second generation immunoassay. One-third of the patients had a history of blood transfusion and about half of the patients did not have an obvious source of HCV infection.

Genotyping Procedures

Viral sequences in sera of infected subjects were typed by one or two techniques:

(1) DNA enzyme immunoassay (DEIA). Viral RNA was reverse transcribed and amplified with primers: p874A: 5′-A[A,G]GAAGATAGA[A,G]AA[A,G]GAGCAACC-3′ (RT and PCRI); p417S: 5′-GG[C,T]GG-[C,T]GG[A,G,C,T]CAGATCGTTGG-3′ (PCRI); p439S: 5′-GAGT[A,T]TAC[G,T,C]TG[C,T]TGCCGCGCAG-3′ (PCRII); and p1AS: 5′-AT[A,G]TACCCCATGAG-[A,G]TCGGC-3′ (PCRII). The resulting DNA fragments were analysed by DEIA as described elsewhere [Viazov et al., 1994].

(2) PCR with a set of type-specific primers. Viral sequences were transcribed and amplified with primers p874a and p417s and subjected to genotyping with type-specific primers according to the established protocol [Widell et al., 1994].

Sequencing and Phylogenetic Analysis

Viral sequences, corresponding to a part of the HCV core gene (nt 439–751, numbering according to Choo et al., 1991), were amplified by RT-PCR with a set of primers p874a, p417s, 1AS, and p439s. The PCR products were purified from agarose gel by QIAquick Gel Extraction Kit (QIAGEN, Germany) and subjected to direct sequencing from both directions using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Sequences of a 216 nt DNA fragment (nt 461 to 676; numbering according to Choo et al., 1991) were subjected to phylogenetic analysis using the package PHYLIP, version 3.5c [Felsenstein, 1993]. Distances between pairs of sequences were estimated using the DNADIST program; phylogenetic trees were constructed using the neighbor-joining algorithm on the previous sets of pairwise distances using the program NEIGHBOR. Significance of the phylogenetic relationships were confirmed by bootstrap resampling (1000 bootstrap replicates) with programs SEQBOOT and CONSENSE. The following HCV sequences from the EMBL database were used (accession numbers): 1a—M62321; 1b—D00574; D10750; D16697; D16698; D16722; D26383; D26384; D30613; D90208; L02836; M58385; M86779; S72728; U63376; U63379; X61594; X76408; X76409; X91302; X91304; X91305; Z29445; Z29446; Z29450; Z29451; Z29452; Z29453; Z29454; 1c—D16189; 1d—D26383; 1e—L38349; 1f—L38350; 2a—D00944; 2b—D10077; 2c—L38337; 2h—Z29455; 2i—X76411; 3a—D17763; 3b—D11443; 4a—Z29466; 5a—Z29472; 6a—U10198.

RESULTS

Seventy-nine randomly chosen HCV RNA positive specimens were tested by DEIA. Twenty-five samples that included all three major HCV types 1a, 1b, 2a, and 3a, were retested by PCR with type-specific primers. In all cases, an absolute concordance was observed between the results of two typing procedures. In all subsequent experiments only the PCR based assay with type-specific primers was used as it was cheaper and easier to perform in our laboratories.

The two genotyping procedures (79 samples were tested by DEIA and 143 by PCR-based technique) allowed us to genotype HCV in all but one of 197 samples (R150) (Tables I and II). Only three major types were identified in this study population. In all four countries HCV 1b was found to be the predominant virus type both in blood donors and in chronic hepatitis patients, followed by 3a, 2a, and 1a. No significant differences in genotype distribution were observed between different countries or between blood donors and chronic hepatitis patients within the same country. Not a single specimen was found to contain more than one HCV type or subtype.

One sample (R150), positive in diagnostic PCR with 5′-UTR primers, was found untypable both by DEIA and the PCR based technique. Sequencing of 216 bp fragment of the core gene demonstrated relatedness of R150 to 2c prototype (Fig. 1).

In order to confirm the genotyping results, 216 bp fragments, corresponding to a part of the HCV core gene, for 40 randomly chosen samples were subjected to direct sequencing and subsequent phylogenetic analysis (Fig. 1). The resulting data demonstrated the clustering of all these subsequences with the appropriate reference sequences (prototypes), thus fully con-

TABLE II. HCV Genotypes in Blood Donors From the Former Soviet Union

		HCV types				
Country	n	1a	1b	2a	2c	3a
Russia	31	—	21 (68%)	4 (13%)	—	6 (19%)
Belaris	21	—	17 (81%)	—	—	4 (19%)
Moldova	14	—	13 (93%)	—	—	1 (7%)
Total	66	—	51 (77%)	4 (6%)	—	11 (17%)

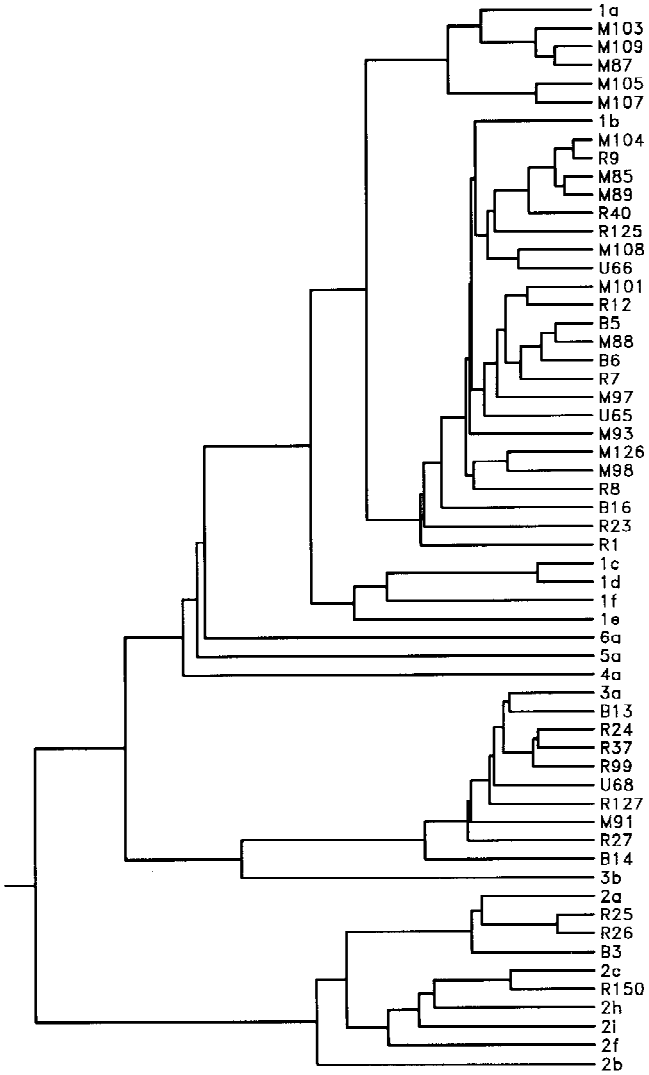

Fig. 1. Phylogenetic analysis of a partial core sequences of HCV isolates from the former Soviet Union. R1, R7, etc., B5, B6, etc., M88, M93, etc., and U65, U66, etc. are sequences of HCV isolates from Russia, Belarus, Moldova, and Uzbekistan, respectively. The following sequences from EMBL database were used as a subtype prototypes: 1a—M62321; 1b—D90208; 1c—D16189; 1d—D26383; 1e—L38349; 1f—L38350; 2a—D00944; 2b—D10077; 2c—L38337; 2f—Z29457; 2h—Z29455; 2i—X76411; 3a—D17763; 3b—D11443; 4a—Z29466; 5a—Z29472; 6a—U10198.

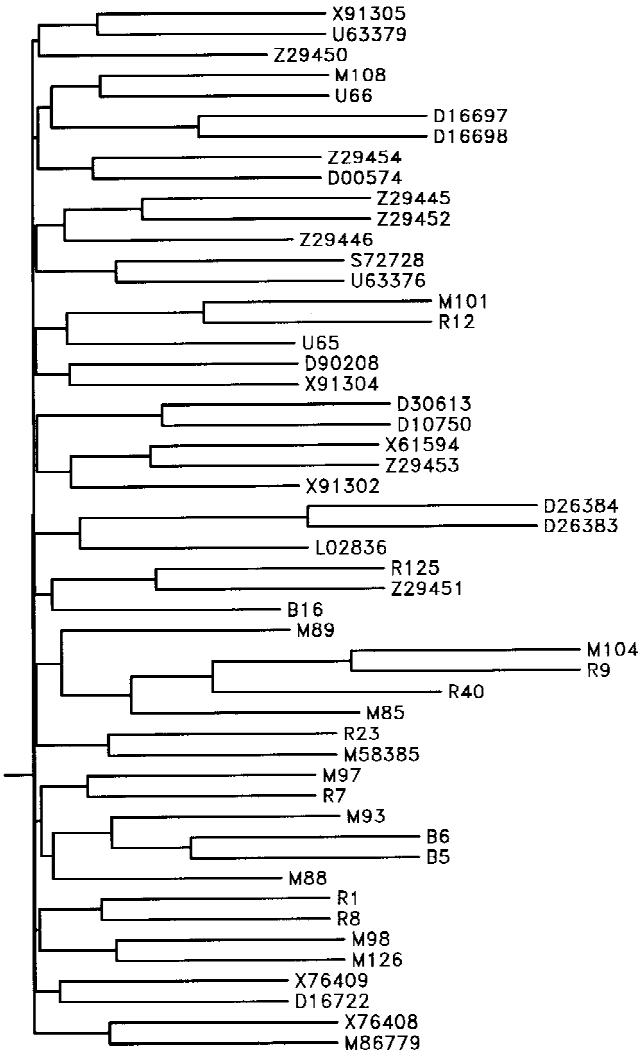

Fig. 2. Phylogenetic analysis of a partial core sequences of HCV 1b isolates from former Soviet Union and others countries. R1, R7, etc., B5, B6, etc., M88, M93, etc., and U65, U66, etc., are sequences of HCV isolates from Russia, Belarus, Moldova and Uzbekistan, respectively. Sequences of HCV isolates from the following countries and regions are indicated by their Genebank admission numbers: Belgium (Z29445, Z29446, Z29452, Z29453); China (L02836, S72728, U63376, U63379); France (X76408, X76409); Germany (M86779); Holland (Z29446, Z29450, Z29454); India (X91302, X91304, X91305); Indonesia (D26383, D26384); Italy (D16698); Japan (D00574, D10750, D30613, D90208, M58385, X61594); Saudi Arabia (D16722) and West India (D16697).

firming the genotype assignment based on the genotyping results.

Two hundred sixteen bp sequences of 23 HCV 1b isolates from this study, along with a set of 28 randomly chosen type 1b sequences taken from the EMBL database, were subjected to bootstrapping and phylogenetic analysis in order to identify a possible split between sequences from the republics of the former Soviet Union and those from other countries (Fig. 2). For most sequences the bootstrap resampling showed grouping with values ranging from 4 to 63 per 100 replicates; for only the fork between sequences D26383 and D26384 was the bootstrap value greater than the generally considered cut-off value of 75%. Thus this analysis did not reveal a segregation of any group of sequences from NIS from the majority of sequences of European, American, or Asiatic HCV isolates.

DISCUSSION

The presented data demonstrate a relatively restricted range of HCV genotypes in the population of the former Soviet Union. Only three major HCV types were identified, and only one specimen was found untypable by both genotyping procedures used. To some extent this genotype distribution is similar to that in Western Europe and North America, where types 1, 2, and 3 account for almost all cases of HCV infection [Bukh et al., 1995; Simmonds, 1995]. Interestingly, a little difference in genotype distribution was noted between blood donors and chronic hepatitis patients in Russia, Belarus, and Moldova, and also between blood donors and patients within each of these countries. Although the number of samples analysed was small, tendency for a similar prevalence of subtypes in chronic hepatitis patients was discovered also in Uzbekistan, a country situated in the Asiatic part of the former Soviet Union. Recently, very similar prevalences of HCV genotypes were reported for Lithuania and several regions of Russia [Viazov et al., 1994; Ambrozaitis et al., 1995; Lvov et al., 1996]. Most probably these similarities reflect the fact of population migration and of intensive contacts between population groups from different parts of Soviet Union as well as the distribution of blood and blood components throughout the country.

Very important is the high predominance of HCV type 1b in all these countries. Several studies [review by Silina and Mondelli, 1995; Simmonds, 1995] provided convincing evidence that infection with HCV 1b, rather than with other types and subtypes, usually has a more aggressive clinical course and that subtype 1b is often associated with the development of hepatocellular carcinoma in non-cirrhotic liver. Also, a significant correlation has been noted between HCV 1b infection and unresponsiveness to interferon treatment. All these facts and considerations undoubtedly should be taken into account in the development of therapeutical strategies in this part of the world.

Another interesting peculiarity of the described genotype distribution is the rather high predominance (up to almost 20% in Russia and Belarus) of HCV type 3a in almost all groups studied. Recently, reports from several European countries revealed that this particular genotype is found much more often in intravenous drug users (IVDU) than in general population [Silini and Mondelli, 1995; Simmonds, 1995; Roggendorf et al., 1996]. In our cohorts, however, only 2 out of 10 subjects (for whom this information was available) with HCV 3a had a history of drug abuse. Whatever the original source of infection, our data suggest that HCV 3a spreads rather effectively among general populations of these countries. Comparison of genotype distribution revealed the presence of a few cases of HCV 1a among chronic hepatitis patients and absence of this subtype among blood donors. Analysis of the available data on the age of individuals under study demonstrated that 86% of subjects infected with HCV 1a were 15 to 18 years old, while the majority of blood donors and chronic hepatitis patients with HCV 1b or 3a were 30 years of age and older. Recently, a relatively high prevalence of HCV 1a (25%) among infected children from Russia was reported [Viazov et al., 1994]. These results probably indicate that HCV 1a was introduced into the former Soviet Union only recently. Higher prevalence of subtype 1a infections among younger patients was reported from several European countries [Simmonds, 1995; Silini and Mondelli, 1995; S. Viazov, unpublished] and possibly reflects changes in HCV prevalence within a particular geographical area over time.

Recently, the time of divergence of variants of subtype 1b was estimated to have occurred 70 to 80 years ago [Smith et al., 1997]. Considering the isolation of the Soviet Union from the rest of the world after the Bolshevik revolution in 1917, one might expect that HCV isolates from Russia and other republics of the former Soviet Union could differ in their sequences from strains circulating in other countries. This supposition, however, was not confirmed by phylogenetic analysis of HCV 1b sequences from this study and randomly chosen 1b sequences from the EMBL database. These findings may indicate that the time of divergence of variants of subtype 1b probably exceeds 80 years.

Noteworthy is the fact that not a single specimen out of 197 contained more than one HCV type or subtype. This observation complements the result of our recent study, which also did not reveal the simultaneous presence of two or more HCV types in any out of 40 samples from multiple exposed subjects, such as hemophiliacs, IVDUs, and haemodialysis patients (S. Viazov, in preparation). Taken together, these data suggest that mixed infection with two types of HCV probably is a very rare event.

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REFERENCES

Ambrozaitis A, Zhagminas K, Balciunaite G, Widell A (1995): Hepatitis C in Lithuania: Incidence, prevalence, risk factors and viral genotypes. Clinical and Diagnostic Virology 4:273–281.

Bukh J, Miller RH, Purcell RH (1995): Genetic heterogeneity of hepatitis C virus: Quasispecies and genotypes. Seminars in Virology 15:41–63.

Choo Q-L, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina-Selby A, Barr PJ, Weiner AJ, Bradley DW, Kuo G, Houghton M (1991): Genetic organisation and diversity of the hepatitis C virus. Proceedings of the National Academy of Sciences, USA 88:2451–2455.

Felsenstein J, PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA.

Kanai K, Kako M, Okamoto H (1992): HCV genotypes in chronic hepatitis C and response to interferon. Lancet 339:1543.

Lamballerie X, Charrel RN, Attoui H, De Micco P (1997): Classification of hepatitis C virus variants in six major types based on analysis of the envelope 1 and nonstructural 5B genome regions

and complete polyprotein sequences. Journal of General Virology 78:45–51.

Lvov DK, Samokhvalov EI, Tsuda F, Selivanov NA, Okamoto H, Stachanova VM, Shakhgildyan IV, Doroshenko IV, Yashina TL, Kuzin SN, Suetina IA, Deryabin PG, Ruzaeva LA, Bezdodov VN, Firsova LA, Sorinson SN, Mishiro S (1996): Prevalence of hepatitis C virus and distribution of its genotypes in Northern Eurasia. Archive of Virology 141:1613–1622.

Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P, The International HCV Collaborative Study Group (1995): Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. Journal of General Virology 76:2493–2507.

Pozzato G, Moretti M, Franzin F, Croce LS, Tiribelli C, Masayu T, Kaneko S, Unoura M, and Kobayashi K (1991): Severity of liver disease with different hepatitis C viral clones. Lancet 338:509.

Roggendorf M, Lu M, Meisel H, Riffelmann M, Schreier E, Viazov S (1996): Rational use of diagnostic tools in hepatitis C. Journal of Hepatology 24:26–34.

Silini E, Mondelli MU (1995): Significance of hepatitis C virus genotypes. Viral Hepatitis Review 1:111–120.

Simmonds P (1995): Variability of hepatitis C virus. Hepatology 21: 570–583.

Simmonds P, Mellor J, Sakuldamrongpanich T, Nuchaprayoon C, Tanprasert S, Holmes EC, Smith DB (1996): Evolutionary analysis of variants of hepatitis C virus found in Southeast Asia: Comparison with classifications based upon sequence similarity. Journal of General Virology 77:3013–3024.

Smith DB, Pathirana S, Davidson F, Lawlor E, Power J, Yap PL, Simmonds P (1997): The origin of hepatitis C virus genotypes. Journal of General Virology (in press).

Viazov S, Kuzin S, Orlova N, Shachgildiyan I, Andriuza K, Paladi N (1992): Prevalence of antibodies to hepatitis C virus in different population groups of the USSR. Problems of Virology 5:366–369 (in Russian).

Viazov S, Zibert A, Ramakrishnan K, Widell A, Cavicchini A, Schreier E, Roggendorf M (1994): Typing of hepatitis C virus isolates by DNA enzyme immunoassay. Journal of Virological Methods 48: 81–92.

Widell A, Mansson A-S, Sundström G, Hansson B-G, Nordenfelt E (1991): Hepatitis C virus RNA in blood donor sera detected by the polymerase chain reaction: comparison with supplementary hepatitis C antibody assays. Journal of Medical Virology 35:253–258.

Widell A, Shev S, Mansson S, Zhang Y-Y, Foberg U, Norkrans G, Fryden A, Weiland O, Kirkus J, Nordenfelt E (1994): Genotyping of hepatitis C virus isolates by a modified polymerase chain reaction assay using type specific primers: epidemiological applications. Journal of Medical Virology 44:272–279.